DESCRIPTION

METHOD OF CONSTRUCTING HOST AND METHOD OF PRODUCING HETEROLOGOUS PROTEIN

TECHNICAL FIELD

The present invention relates to a eukaryotic host microorganism in which part of the genome of the eukaryotic microorganism is modified for the purpose of improving the productivity of a heterologous protein by a transformant of the eukaryotic host microorganism, a method of constructing the host, a trasnformant of the host and a method of producing a protein using the transformant. The eukaryotic microorganism is preferably the fission yeast, *Schizosaccharomyces pombe* (hereinafter referred to as *S. pombe*).

BACKGROUND ART

Recombinant DNA technology is used for production of heterologous proteins in various host microorganisms and animals including *Escherichia coli* (hereinafter referred to as *E. coli*). The target products are various biogenous proteins (herein, inclusive of polypeptides), and many of them have already been produced industrially for medical and other uses so far.

Among various hosts developed for production of heterologous proteins, yeasts seem favorable for expression of animal and plant proteins because of their eukaryotic similarity in the transcription and translation systems to animals and plants, and the

baker's yeast (*Saccharomyces cerevisiae*) is a widely used host. Among yeasts, *S. pombe* is known to be close to animal cells in nature as is evident from the fact that it grows by fission not by budding as a result of the different evolution process it has followed since it diverged from other yeasts at early stages. Therefore, the use of *S. pombe* as the host for expression of heterologous proteins is expected to provide a gene product closer to its natural form in animal cells.

Though studies of gene expression in *S. pombe* is delayed, the recent discovery of potent promoters functional in *S. pombe* has accelerated the development of expression systems using *S. pombe* as the host, and various improvements have been added to expression vectors to develop more stable and efficient expression systems (Japanese Patent No. 2776085, JP-A-07-163373, JP-A-10-215867, JP-A-10-234375, JP-A-11-192094, JP-A-2000-136199, JP-A-2000-262284). As a result, expression systems using *S. pombe* as the host show high production efficiency now.

Production systems for heterologous proteins using eukaryotic microorganisms such as yeasts can be realized easily by conventional microbiological techniques and recombinant DNA technology with high productivity. Large cultures are already available and are acceleratingly used for actual production. Even after the scale is enlarged for actual production, cells retain the high

production efficiency per cell obtained in the laboratory.

However, considering that cost reduction is often demanded in actual production, it is necessary to improve the production efficiency of heterologous proteins through improvement in cell growth efficiency, suppression of degradation of the heterologous protein of interest, more efficient eukaryotic modifications in the microorganisms or more efficient utilization of the nutrition sources. For example, increase in the conversion of the carbon sources added to the medium for culture growth into the heterologous protein of interest is expected to drastically improve cell growth efficiency and therefore production efficiency of the heterologous protein, because efficient utilization of the carbon sources in the medium for production of the heterologous protein of interest seems to be sacrificed for their consumption by metabolic systems unnecessary for cell growth or production of the heterologous protein of interest (such as the ethanol fermentation system for production of ethanol).

DISCLOSURE OF THE INVENTION

Under the above-mentioned circumstance, the present inventors studied from the above-mentioned aspects, and, as a result, found that the deletion or inactivation of part or all of the genome of the host unnecessary or detrimental to production of the heterologous protein by its transformant improves the production efficiency of

the heterologous protein. The present invention aims at improvement in the production efficiency of a heterologous protein, relates to a method of constructing a eukaryotic host organism, a host constructed by the construction method, a transformant of the host obtained by introducing a gene encoding a heterologous protein into the host and a method of producing a heterologous protein using the transformant, and provides:

(1) a method of constructing a eukaryotic host microorganism for production of a heterologous protein encoded by a transgenically introduced gene, which is characterized by deleting or inactivating part or all of the genome of a eukaryotic host microorganism unnecessary or detrimental to production of the heterologous protein by a transformant of the host in culture for the purpose of improving productivity of the heterologous protein;

(2) a eukaryotic host microorganism for production of a heterologous protein encoded by a transgenically introduced gene, which is constructed by the construction method;

(3) a transformant obtained by introducing the structural gene encoding a heterologous protein into a eukaryotic host microorganism in which part or all of the genome of the eukaryotic host microorganism unnecessary or detrimental to production of the heterologous protein by the transformant in culture has been deleted or inactivated for the purpose of improving productivity of

the heterologous protein; and

(4) a method of producing a heterologous protein, comprising causing a transformant of a eukaryotic host microorganism having a gene encoding a heterologous protein extrinsic to the host and collecting the heterologous protein, wherein the productivity of the heterologous protein is improved by deleting or inactivating part or all of the genome of the eukaryotic host microorganism which is unnecessary or detrimental to production of the heterologous protein by the transformant in culture.

The part of the genome unnecessary or detrimental to production of the heterologous protein by the transformant in culture is preferably genes associated with energy metabolism, proteases, meiosis, transcription, cell growth and division and DNA synthesis, protein synthesis, membrane transport, cell structure maintenance, signal transduction or ion homeostasis in the eukaryotic host microorganism.

The eukaryotic microorganism is preferably a yeast, especially *S. pombe*. The part of the genome unnecessary or detrimental to production of the heterologous protein by *S. pombe* is a gene selected from the genes associated with energy metabolism (such as pyruvate decarboxylase gene) and the genes associated with proteases (such as endopeptidases like serine protease gene and exopeptidases like aminopeptidase and carboxypeptidase).

BEST MODE OF CARRYING OUT THE INVENTION

In the present invention, the eukaryotic microorganism is preferably a fungus, especially a unicellular fungus (i.e., a yeast). As the yeast, a yeast of the *Saccharomyces* genus such as the baker's yeast, a yeast of the *Shizosaccharomyces* genus or a yeast of the *Pichia* genus is preferable. Eukaryotic microorganism of the *Aspergillus* genus, the *Rhizopus* genus or the *Penicillium* genus and other eukaryotic microorganism may be mentioned. The eukaryotic microorganism particularly preferred in the present invention is a yeast of the *Schizosaccharomyces* genus, especially *S. pombe*. Hereinafter, hosts mean those eukaryotic microorganisms, unless otherwise noted.

It is common in recent years to transgenically introduce the gene encoding a protein extrinsic to a host (i.e., a heterologous protein) (hereinafter referred to as a heterologous gene) to the host and causing the host having the introduced heterologous gene (i.e., a trasnformant) to produce the heterologous protein and collecting the heterologous protein. While the culture of the transformant is producing the heterologous protein, part of the genome is unnecessary or detrimental to production of the heterologous protein by the transformant in culture. The part of the genome may be a gene or a nongenomic part, preferably a genomic part of the genome. Deletion or inactivation of the gene

improves the production efficiency of the heterologous protein by the transformant. It is believed that a lot of such unnecessary or detrimental genes exist in a genome. Deletion or inactivation of part of these genes sufficiently meets the purpose of the present invention.

The part of the genome unnecessary or detrimental to production of the heterologous protein by the transformant may be genes essential for the wild type host to survive or grow, because such essential genes are not always necessary to a transformant culture. For example, the genes essential for conversion of carbon sources to nutrients are no longer necessary if the nutrients are added to the culture environment (culture medium). Meanwhile, in the case of yeasts which can grow not by meiosis but by budding or fission, genes associated with meiosis are not always necessary for the growth of a transformant. The existence of such unnecessary genes can be a burden to growth of the transformant or production of the heterologous protein. Therefore, deletion or inactivation of such genes lightens the burden and improves the production efficiency of the heterologous protein.

On the other hand, genes associated with proteases tend to inhibit the production of the heterologous protein. Because the heterologous protein produced is fundamentally unnecessary to the host, the transformant tends to degrade the produced heterologous protein by

proteases. Since degradation of the heterologous protein is considered as a factor of reduction in the production efficiency of the heterologous protein, deletion or inactivation of the genes associated with production of such proteases improves the production efficiency of the heterologous protein.

Such genes unnecessary or detrimental to production of the heterologous protein as described above are preferably genes associated with energy metabolism, proteases, meiosis, transcription, cell growth and division and DNA synthesis, protein synthesis, membrane transport, cell structure maintenance, signal transduction or ion homeostasis. Particularly preferred are genes selected from the genes associated with energy metabolism and the genes associated with proteases.

The gene in the genes associated with energy metabolism is a gene associated with ethanol fermentation. A typical example of the genes associated with ethanol fermentation is the gene encoding pyruvate decarboxylase (the pyruvate decarboxylase gene). Deletion or inactivation of the pyruvate decarboxylase gene is considered to make the culture of the transformant distribute more energy to synthetases instead of ethanol fermentation and thereby improve the production efficiency of the heterologous protein.

The genes associated with proteases include genes encoding endopeptidases such as serine proteases,

carboxyl proteases and metal proteases and exopeptidases such as aminopeptidases and carboxypeptidases. Particularly preferred are genes encoding serine proteases (serine protease genes), genes encoding aminopeptidases (aminopeptidase genes) and genes encoding carboxypeptidases (carboxypeptidase genes). Deletion or inactivation of these genes associated with proteases is considered to improve the production efficiency of the heterologous protein.

Part of the genome of the host can be deleted or inactivated by known methods. One or more parts of the genome may be deleted or inactivated. When the part to be deleted or inactivated is gene(s), deletion or inactivation a single gene or at least two genes may be effected on a single gene or two or more individual genes.

Deletion of a gene may be deletion of the entire gene or deletion of part of the gene for inactivation of the gene. Inactivation of a gene means not only deletion of part of the gene but also modification of the gene without deletion. A gene may be inactivated by inserting another gene or DNA into a certain sequence in the gene as the inactivation target. In any case, the target gene is inactivated so as to encode an inactive protein or so as to be unable to be transcribed or translated.

Though there is no restriction on the heterologous protein, it is preferably a protein which is produced by multicellular organisms such as animals and plants,

especially a protein produced by a mammal (inclusive of human). Such a protein is rarely obtained with high activity by a prokaryotic host microorganism such as E. coli and is obtained with low production efficiency by using an animal cell line such as CHO as the host. The use of the transgenic eukaryotic host microorganism of the present invention is considered to solve these problems.

EXAMPLES

Now, the present invention will be described in further detail in reference to specific Examples.

Example 1 Improvement in the production efficiency of Aequorea victria green fluorescent protein by inactivation of the pyruvate decarboxylase gene pdc1

A 1.8-kb fragment from the orotidine phosphate decarboxylase gene was inserted in the 1785-bp ORF (the protein-coding region) of the pyruvate decarboxylase gene pdc1 (SPAC1F8.07) of the fission yeast *S. pombe* to obtain a pdc1-disrupted vector. A green fluorescent protein-producing uracil-requiring auxotroph (obtained by inactivating the orotidine phosphate decarboxylase activity of the yeast strain used in the octuplicated integrative production system disclosed in JP-A-2000-262284 through gene disruption) was transformed with the vector. A uracil-unrequiring strain capable of forming colonies on the minimum medium was collected. Analysis of the genomic DNA by PCR amplification confirmed

disruption of the pyruvate decarboxylase gene.

The transformant was grown and tested for green fluorescent protein production in YPD liquid medium (1% yeast extract (DIFCO), 2% Bacto-Peptone (DIFCO), 2% glucose (Wako Pure Chemical Industries, Ltd.)) in test tube-shaped culture vessels. The production per cell was higher than in the original strain, according to fluorometry using a microplate reader (CORONA, MTP-32+MTPF2) at an excitation wavelength of 490 nm and an emission wavelength 530 nm.

Example 2 Improvement in the production efficiency of *Aequorea victria* green fluorescent protein by inactivation of the serine protease gene isp6

A 1.8-kb fragment from the orotidine phosphate decarboxylase gene was inserted in the ORF (1404 bp) of a serine protease gene isp6 (SPAC4A8.04) of the fission yeast *S. pombe* to obtain a isp6-disrupted vector. The same uracil-requiring auxotroph as in Example 1 was transformed with the vector. A uracil-unrequiring strain capable of forming colonies on the minimum medium was collected. Analysis of the genomic DNA by PCR amplification confirmed disruption of the serine protease gene.

The transformant was grown in the same manner as in Example 1 and tested for green fluorescent protein production. The production per cell was higher than in the original strain, according to fluorometry using a

microplate reader (CORONA, MTP-32+MTPF2) at an excitation wavelength of 490 nm and an emission wavelength 530 nm.

Example 3 Improvement in the production efficiency of Aequorea victria green fluorescent protein by inactivation of the aminopeptidase gene SPC4F10.02 (aminopeptidase I)

400-bp genomic DNA sequences franking the ORF (1500 bp) of the aminopeptidase gene SPC4F10.02 (Nature 415, 871-880 (2002)) from the 5'- and 3'-sides were prepared by PCR amplification using primers having the nucleotide sequences ACAAGCAGATCTCCCAGTCA and AGCCAGTGGGATTTGTAGCTTTTTCCATGTAATTGCATTT and the nucleotide sequences AAAAGTTTCGTCAATATCACTTTACCAAGTTTGTTTATGT and GCTTTCGTTGAAAGACTTG. Then, these DNA fragments were ligated with a 1.8-kbp fragment from the ura4 gene as the marker gene by PCR amplification using primers having the nucleotide sequences ACAAGCAGATCTCCCAGTCA and GCTTTCGTTGAAAGACTTG to give a gene disruptive vector having the ura4a gene instead of the ORF of the aminopeptidase gene in the genomic DNA sequence.

A *S. pombe* strain (leu1-32, ura4-D18) was transformed with the vector. A uracil-unrequiring strain capable of forming colonies on the minimum medium was collected. Analysis of the genomic DNA using PCR amplification designed so as to amplify DNA fragments only when the intended gene was disrupted confirmed disruption of the

aminopeptidase gene SPC4F10.02.

The transformant was transformed with an expression vector obtained by inserting the *Aequorea victria* green fluorescent protein gene in the expression vector for fission yeast (JP-A-7-163373). After screening, the resulting transformant was incubated in YPD liquid containing 100 mg/L antibiotic G418 as the expression medium in test tube-shaped culture vessels. The production per cell was higher than in the original strain. Namely, according to fluorometry using a microplate reader (CORONA, MTP-32+MTPF2) at an excitation wavelength of 490 nm and an emission wavelength 530 nm, the expression level was increased about twice to 231, as compared with that by the non-disruptive strain containing the same gene insert.

Example 4 Improvement of the production efficiency of *Aequorea victria* green fluorescent protein by inactivation of the carboxypeptidase SPBC16G5.09

400-bp genomic DNA sequences franking the ORF (1647 bp) of the carboxypeptidase gene SPBC16G5.09 (Nature 415, 871-880 (2002)) from the 5'- and 3'-sides were prepared by PCR amplification using primers having the nucleotide sequences CGTATTAGCGATTGAACTG and AGCCAGTGGGATTTGTAGCTGCTCTCACAATCAAATCGAC and the nucleotide sequences AAAAGTTTCGTCAATATCACACTGTATATAAATCTTTTCT and CAGGGAAGAACGTTCCAAGA. Then, these DNA fragments were

ligated with a 1.8-kbp fragment from the ura4 gene as the marker gene by PCR amplification using primers having the nucleotide sequences CGTATTAGCGATTGAACTG and CAGGGAAGAACGTTCCAAGA to give a gene disruptive vector having the ura4 gene instead of the ORF of the aminopeptidase gene in the genomic DNA sequence.

A *S. pombe* strain (leu1-32, ura4-D18) was transformed with the vector. A uracil-unrequiring strain capable of forming colonies on the minimum medium was collected. Analysis of the genomic DNA using PCR amplification designed so as to amplify DNA fragments only the intended gene was disrupted confirmed disruption of the carboxypeptidase SPBC16G5.09.

The transformant was transformed with an expression vector obtained by inserting the *Aequorea victria* green fluorescent protein gene in the expression vector for fission yeast (JP-A-7-163373). After screening, the resulting transformant was incubated in YPD liquid containing 100 mg/L antibiotic G418 as the expression medium in test tube-shaped culture vessels. The production per cell was higher than in the original strain. Namely, according to fluorometry using a microplate reader (CORONA, MTP-32+MTPF2) at an excitation wavelength of 490 nm and an emission wavelength 530 nm, the expression level was increased by about 1.6 times to 215, as compared with that by the non-disruptive strain containing the same gene insert.

Example 5

400-bp genomic DNA sequences franking the ORFs of various genes (Nature 415, 871-880 (2002)) from the 5'- and 3'-sides were prepared by PCR amplification using primers (four types A-D). Then, these DNA fragments were ligated with a 1.8-kbp fragment from the ura4 gene as the marker gene by PCR amplification using two primers (A and D of the above-mentioned four) to give gene disruptive vectors having the ura4 gene instead of the ORFs of the genes in the genomic DNA sequence. A *S. pombe* strain (leu1-32, ura4-D18) was transformed with the vectors. Uracil-unrequiring strains capable of forming colonies on the minimum medium were collected. Analysis of the genomic DNA using PCR amplification designed so as to amplify DNA fragments only when the intended genes were disrupted confirmed disruption of the intended genes.

The gene-disruptive *S.pombe* transformants were transformed with an expression vector obtained by inserting the *Aequorea victria* green fluorescent protein gene in the same manner as in Examples 3 and 4. The transformants were incubated, and the green fluorescent protein productions were measured at an excitation wavelength of 490 nm and an emission wavelength 530 nm. The absorbances were higher than those of non-disruptive strains containing the same genes insert.

The disrupted genes and the primers (4 types) used are given below.

(1)

The name of the gene: Aspertic protease gene SPCC1795.09 (putative aspartic proteinase)

The length of the ORF: 1566 bp

Primers:

A; TTCATCTCGGACGTGTAG

B; AGCCAGTGGGATTTGTAGCTTTAATTAAATGTGTATTTTA

C; AAAAGTTTCGTCAATATCACATCCTTAAATAATTAGAAGA

D; TCCACTTTCTGTTGTGGA

(2)

The name of the gene: Cytoplasmic aminopeptidase gene SPAC13A11.05 (cytosol amino peptidase)

The length of the ORF: 1542 bp

Primers:

A; AATCTGCAATCGGACATCGC

B; AGCCAGTGGGATTTGTAGCTTGTACGTAAGAAAAAAAGCT

C; AAAAGTTTCGTCAATATCACCTTATTTATTTTCTTGGCTA

D; CAACATGAGACTTCAACCGA

(3)

The name of the gene: Dipeptidyl aminopeptidase gene SPC14C4.15 (dipeptidyl aminopeptidase)

The length of the ORF: 2606 bp

Primers:

A; GGCCCATTAGCTATATGAGAC

B; AGCCAGTGGGATTTGTAGCTAATAGAAAAGTTACGTTATT

C; AAAAGTTTCGTCAATATCACTCATGCCACTGGAATAAGTG

D; TACCCACCAACTTATAAGCC

(4)

The name of the gene: Carboxypeptidase gene SPBC337.07c (putative carboxypeptidase)

The length of the ORF: 1665 bp

Primers:

A; GACTATGTTGGTGGAGTGCAA

B; AGCCAGTGGGATTTGTAGCTTCCAAGAAAGATCAATAATT

C; AAAAGTTTCGTCAATATCACGAGTTAGAAAGAGCAGTCTT

D; TAGGCAATAGTGAGACCTGA

(5)

The name of the gene: Vacuolar carboxylase S gene SPAC24C9.08 (putative vacuolar carboxypeptidase s)

The length of the ORF: 1791 bp

Primers:

A; TCAGGTGTCATCACTCAC

B; AGCCAGTGGGATTTGTAGCTTGTCGTAGTTTTAGAAATTA

C; AAAAGTTTCGTCAATATCACGCTCCTTTTTTGGATTTGCT

D; CCCTTCTAAACATACTACACGTTC

(6)

The name of the gene: Zinc protease gene SPCUNK4.12c (putative zinc-protease)

The length of the ORF: 2910 bp

Primers:

A; TCTGGAAAATTGCTCGTTAG

B; AGCCAGTGGGATTTGTAGCTTTTTTATTTATGAAAGGAAA

C; AAAAGTTTCGTCAATATCACTTTTTTTTCCCTAATCCGAT

D; TGCAAGACTCCAATGCTC

(7)

The name of the gene: Zinc protease gene SPCC1442.07c (putative ZN-protease)

The length of the ORF: 849 bp

Primers:

A; TCCACCCTTTGTCCATGA

B; AGCCAGTGGGATTTGTAGCTTGGATTCTTTACTACTTATA

C; AAAAGTTTCGTCAATATCACGTGAATTTGGTAATTAGCAA

D; CTGGCTGTTCTTAGTCAG

(8)

The name of the gene: Metalloprotease gene SPCC965.04c (putative metallopeptidase)

The length of the ORF: 2231 bp

Primers:

A; ACGATTTTCCACTTGTCCA

B; AGCCAGTGGGATTTGTAGCTGCCAAGACTGTTAGAGTCAT

C; AAAAGTTTCGTCAATATCACAAATTTTGCAATACAAAAAG

D; TCAGGATATCGCTGTCACT

(9)

The name of the gene: Zinc metalloprotease gene SPAC17A5.04c (putative zinc metallopeptidase)

The length of the ORF: 1610 bp

Primers:

A; GGGTACTCTCAAGAAGGATGT

B; AGCCAGTGGGATTTGTAGCTACGCCTTTTCGTTTCTTTTG

C; AAAAGTTTCGTCAATATCACAGTATATCATATATTCTTTT

D; ATCCTTGGGTACGCGTAA

(10)

The name of the gene: CAAX prenyl protease I gene SPC3H1.05 (putative CAAX prenyl protease)

The length of the ORF: 1495 bp

Primers:

A; GTTGTTGATGCAACGGCTAA

B; AGCCAGTGGGATTTGTAGCTAAATAGAGTTCAACTATCGA

C; AAAAGTTTCGTCAATATCACGTTTCATGAGTGAATGAAAT

D; TATGCTCATACGTTCCCT

(11)

The name of the gene: Dipeptidyl peptidase gene SPBC1711.12 (putative dipeptidyl peptidase)

The length of the ORF: 2052 bp

Primers:

A; GTTTTGTTGAGATGTCTTGG

B; AGCCAGTGGGATTTGTAGCTCCAAAAAAATATATTCTTTG

C; AAAAGTTTCGTCAATATCACATTAATTTTAATAATACAAC

D; GAATCTCGTATTCCGGCATT

(12)

The name of the gene: Dipeptidase gene SPCC965.12 (putative dipeptidase)

The length of the ORF: 1251 bp

Primers:

A; CGCTGTGCTAATCAACTG

B; AGCCAGTGGGATTTGTAGCTTTTCAACTATTATCAGCTTC

C; AAAAGTTTCGTCAATATCACTATCATAAGGATCGTTGACT

D; ACACAATGTGGATACGAACT

(13)

The name of the gene: Methionine metallopeptidase gene SPBC 14C8.03 (putative methionine metallopeptidase)

The length of the ORF: 1281 bp

Primers:

A; GTTGCTTGATATCCGACTCA

B; AGCCAGTGGGATTTGTAGCTTGTTTAAGATTGTTAAATCC

C; AAAAGTTTCGTCAATATCACAAAAATTTTTTTTGTGCTGG

D; CCGTTCATCGAATAGCTCAA

(14)

The name of the gene: Methionine aminopeptidase gene SPBC3E7.10 (putative methionine aminopeptidase)

The length of the ORF: 1301 bp

Primers:

A; TCCAAATACCAGCATACGCA

B; AGCCAGTGGGATTTGTAGCTATAAATACTTTGTCTTAAGG

C; AAAAGTTTCGTCAATATCACATTTTGATATACCCAACATG

D; GCGCCAAACGAAAAGAGTGA

(15)

The name of the gene: Signal peptidase gene SPAC1071.04c (putative signal peptidase)

The length of the ORF: 504 bp

Primers:

A; TCCATAGCATGATTAGGCAA

B; AGCCAGTGGGATTTGTAGCTTTGAGCTCAATTTTTTTAAT

C; AAAAGTTTCGTCAATATCACTTTTACTATTAGCTTAATTA

D; TTCAACAGTCATTGCGATTG

(16)

The name of the gene: Mitochondrial processing peptidase β subunit gene SPBP23A10.15c (mitochondrial processing peptidase beta subunit)

The length of the ORF: 1374 bp

Primers:

A; AGCAACCGACTTTGCACT

B; AGCCAGTGGGATTTGTAGCTACGCATTTTCTTGGGACTTT

C; AAAAGTTTCGTCAATATCACGCATAATCAATTCAAGCTCC

D; CGGTCATTCGTTTCCTTC

Industrial Applicability

Inactivation of the pyruvate decarboxylase gene or a protease gene in the fission yeast *S. pombe* improves production efficiency of a heterologous protein in a transformant of the fission yeast host S. pombe. Thus, in a protein production system using a transformant having a transgenically introduced gene encoding a heterologous protein, deletion or inactivation of part of the genome unnecessary or detrimental to production of the heterologous protein by the transformant in culture improves production efficiency of the heterologous protein.

The entire disclosure of Japanese Patent Application No. 2001-160128 filed on May 29, 2001 including specification, claims and summary is incorporated herein by reference in its entirety.